



Antistreptolysin O Titers in Healthy and Diseased Children in the Fez Region in Morocco

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Abstract

Background: The diagnosis of acute rheumatic fever is based on the modified Jones criteria with an elevated antistreptolysin O titer. The upper limit of normal for the antistreptolysin O titers varies by population. The aim is to establish the upper limit of normal for the antistreptolysin O in healthy and diseased children in the Fez region in Morocco.

Methods: The study was conducted in two pediatric wards and a Healthcare center from 2016 to 2018. We included 447 children who are classified into 5 groups; Group 1: 155 normal children, group 2: 26 children with acute rheumatic fever, group 3: 94 children with rheumatic heart disease, group 4: 101 children with tonsillitis and group 5: 71 children with a history of recurrent tonsillitis.

Results: The upper limit of normal for the antistreptolysin O titer was: group 1; 421.4 IU, group 2; 829.6 IU, group 3; 580.2 IU, group 4; 561.8 IU and group 5; 756.2 IU. Antistreptolysin O titers are high for several months in all groups. For children with rheumatic heart disease, it starts to decrease after 9 months. These children adhere to prophylaxis for several years. High titers in this group confirm that there is no relation between carditis and antistreptolysin O titers. The upper limit of normal is higher in boys with acute rheumatic fever, rheumatic heart disease and history of acute recurrent tonsillitis. It is significantly higher in children age 5 to 15 years during cold periods.

Conclusion: Moroccan children have a high upper limit of normal for the antistreptolysin O exceeding 400 IU that should be taken into consideration when the diagnosis of rheumatic fever.

Keywords: Children; Antistreptolysin O; Upper limit of normal; Acute rheumatic fever; Rheumatic heart disease

Introduction

Group A streptococcus (GAS) tonsillitis is the most common streptococcal throat infection in school-aged children [1,2]. An inadequate treatment of GAS tonsillitis causes acute rheumatic

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fever (ARF) that is rare but a serious post-streptococcal infection. ARF is relatively rare in developed countries, but present a major problem of public health in the developing world [3]. The world health organization (WHO) estimate that 15.6 to 19.6 million people have rheumatic heart disease (RHD), 2.4 million of them are children aged 5 to 14 years and 79% of all RHD cases came from developing countries [4]. There isn't a reporting about the prevalence of ARF in Morocco. However, a recent study in the north of Morocco shows that between 2009 and 2015 a total of 209 children was diagnosed with ARF, 53.1% of them was developed RHD and the mortality rate was 2.7% [5]. The diagnosis of acute rheumatic fever is based on Jones criteria with a proven evidence of prior infection with group A Streptococcus [6]. After infection, antistreptolysin O titers (ASOT) begin to rise by the first week, a maximal response being reached 3 to 6 weeks and the decline may begin 6 to 8 weeks after the infection. Frequently, the serum taken at disease onset may be convalescent and a rising titer can be not demonstrate [7]. Also, a proportion of healthy populations may have elevated values [8]. Although the measure of ASO has helped to diagnose recent infections, a non-group A β -hemolytic streptococci can also cause elevated ASO titers and may falsed the diagnosis. The upper limit of normal (ULN) of ASOT suggested for adults is different to these for children and the laboratory personnel may misinterpret streptococcal antibody titers. The distribution of the ULN values of ASOT varies depending on the population, age, geographic locations, site of infection, and season [9-11]. Since many pediatricians are supporting the diagnosis of ARF on a history of recurrent follicular tonsillitis and a high ASOT, the establish of the ULN value in normal children is necessary to interpret ASOT in cases suspected to have ARF. This is the first study in Morocco that aims to determine the ULN of ASOT in healthy children, in children with ARF, RHD and in those with tonsillitis. The variation of ASOT with season and age was also evaluated.

Subjects and Methods

This is a cross-sectional study conducted on 447 patients chosen consecutively from the pediatric cardiology ward and the pediatric Traumatology ward of the hospital Hassan II center, and the Healthcare center of Narjiss in Fez city in Morocco from January 2016 to December 2018.

Subjects were classified into five groups.

Group 1(control) consisted of 155 children: 131 were healthy children with no history of acute rheumatic fever, recent tonsillitis or sore throat and 24 had non rheumatic heart disease.

Group 2 (ARF): 26 patients with ARF without carditis, diagnosed according to updated modified Jones criteria.

Group 3 (RHD): 94 patients with RHD, all of whom had mitral regurge with or without aortic regurge confirmed by echocardiography and on long-acting penicillin (LAP)

prophylaxis every 3rd week for >1 year. These patients had no history of acute follicular tonsillitis or rheumatic activity within three months prior to enrollment in the study. These three groups were recruited during the consultation in the pediatric cardiology ward of the hospital Hassan II center in Fez city.

Group 4 (acute follicular tonsillitis): 101 children included in this group are those coming to the ANNRJISS health center in Fez and diagnosed with acute follicular tonsillitis by the attending physician. These children presented with erythematous or erythematous-pultaceous sore throat, fever and headache. Throat samples were collected from these children for identification of group A Streptococcus.

Group 5 (history of recurrent follicular tonsillitis): 71 apparently healthy children with past history of repeated follicular tonsillitis more than three times of year for the past 3 years. Children recruitment was carried out in the pediatric Traumatology ward of the hospital Hassan II center. Children with proven attack of acute follicular tonsillitis within the three months before enrollment in the study were excluded.

The studied groups were classified according to gender (male/female). Each group was sub-classified into three subgroups according to age: ≤ 5 , 5-15 and ≥ 15 years. They were also classified according to the season of enrollment into the study: winter, spring, summer, and autumn. A full history was taken of all study subjects and they all underwent clinical examination with a particular emphasis on the presence of acute tonsillar inflammation, the number of attacks of acute follicular tonsillitis per year, criteria of rheumatic activity and RHD. Written information about the nature of the experimental procedures was given to parents of children, who asked for their consent. Blood specimens were collected by venipuncture in clean, sterile and small test tube from all subjects and conserved at 4 degrees. The samples were forwarding of the department of biochemistry of the Laboratory of medical analysis on the hospital Hassan II on the same day. On arrival at the laboratory, the specimens were centrifuged and the serum was used for ASO measurement. The measurement of ASO levels was performed on architect c8000 systems (Abbott, France) and reagents were obtained from Masterlab (Quantia ASO kit). It is a quantitative measurement of ASO concentration carried out by measuring the turbidity of reagents, considering the peak agglutination rate and using known concentration patterns for comparison. The results are automatically calculated through logarithmic function and expressed in international units - IU/mL. The measurement of ASOT is carried out just one time for the control group and every three months for the groups 2, 3 and 5 for one year. Concerning group 4, the measurement of ASOT is done at the moment of the pharyngitis, after one month and after 3 months of the episode of the tonsillitis. All patients performed the analysis in the same laboratory.

Statistical Analysis

The upper limit of normal ASOT was calculated in different groups as the value exceeded by 10% of the studied population (90th percentile). Mean and standard deviation (SD) were calculated for continuous variables. Student’s t test evaluated associations between ASO values and different categorical variables. ANOVA (analysis of variance) was used to test the difference between mean values of ASOT among the five groups, in the different age groups and to explore the association between number of attacks of tonsillitis and values of ASO. Pearson’s correlation was used to investigate the relationship between age and ASO titer, and between the values of ASO in each two groups. The threshold value of significance was set at 0.05. All statistical analyses were conducted using SPSS software v21.

Ethical standards

The study was approved by the Joint Research Ethics Committee of Medical School and the Hassan II University Hospital of Fez, Morocco. The protocol was performed in accordance with the relevant guidelines and regulations/ Declaration of Helsinki.

Results

During the period of study, four hundred forty seven children were included. The mean age and the sex ratio male/female of the different groups were recorded.

Group 1: 10.2 +/- 3,4 years (2.2)

Group 2: 11.7 +/- 3 years (1.2)

Group 3: 12.6 +/- 3 years (0.52)

Group 4: 10.1 +/- 3,6 years (0.74)

Group 5: 9.9 +/- 3, 2 years (0.8)

The data of ASOT in the five groups are presented in the (Table 1,2) (Figure 1).

Table 1: Antistreptolysin O titer levels in the studied groups.

Groups	Controls	ARF	Chronic RHD	Acute follicular tonsillitis	H of acute recurrent f. tonsillitis
ULN (90th percentile)	421.4	829.6	580.2	561.8	756.2
Mean (95% CI)	208.75 (180.65, 236.86)	331.95 (222.22, 441.8)	281.09 (230.19, 331.98)	293.57 (250.02, 337.12)	397.69 (336.20, 459.18)
GMT	158.98	251.67	205.51	227.47	328.66

ARF: Acute Rheumatic Fever; RHD: Rheumatic Heart Disease; H of recurrent F tonsillitis: History Of Recurrent Follicular Tonsillitis; ULN: Upper Limit Of Normal (80th percentile); GMT: Geometric Mean Titer; CI: Confidence Interval

Table 2: Distribution of groups according to ASO class (<200 UI/ mL, 200-400 UI/ mL, >400 UI/ mL).

	<200	200-400	>400
Controls	99	39	17
ARF	9	10	7
Chronic RHD	46	29	19
Acute follicular tonsillitis	36	40	25
H of acute recurrent f. Tonsillitis	13	29	29

Table 3: Variation of the ULN in each studied group as a function of time.

ASO Groups	Time 0	3 months	6 months	9 months	1 year
RHD	748.4	722	555	734.5	549.4
ARF	819,49	841,25		.	
Acute follicular tonsillitis	585,3	684,19	962,80	.	
H of recurrent f. tonsillitis	875,49	755	738		

Controls	421,4				
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Table 4: Antistreptolysin O titer in age based sub-groups.

	N effective	Moyenne ± ecart type	ULN
Control			
<5 years (N)			
5-15 years (N)	150	208,32(177,85)	419.1
>15 years (N)	5	221,8(171,26)	
ARF			
<5 years (N)			
5-15 years (N)	23	325,46 (263,82)	738.6
>15 years (N)	3	381,70(390,85)	
RHD			
<5 years (N)	1	368,67	
5-15 years (N)	70	325,37 (262,48)	641,95
>15 years (N)	23	142,49 (135,21)	307,4
Acute follicular tonsillitis			
<5 years (N)			
5-15 years (N)			
>15 years (N)	94	298,45(223,98)	573,25
	7	228(167,91)	
History of recurrent f. tonsillitis			
<5 years (N)			
5-15 years (N)			
>15 years (N)	69	403,28(260,69)	761
	2	204,75 (160,16)	

ARF: Acute Rheumatic Fever; RHD: Rheumatic Heart Disease; H of recurrent F tonsillitis: History Of Recurrent Follicular Tonsillitis; ULN: Upper Limit Of Normal (80th percentile); GMT: Geometric Mean Titer; CI: Confidence Interval

Table 5: Variation of the ULN of ASO according to the season.

Groups	ARF	Acute follicular tonsillitis	H of recurrent f. tonsillitis	Controls
Autumn	504.8	542,2000	606,5250	571,500
Winter	813.1	542,9000	899,0000	333,000
Spring	656.1	1061,0000	934,5000	768,100
Summer	756.6		462,5000	322,300

Table 6: Association between ASOT and number of attacks of tonsillitis.

N of attack			ASO		
	N	Mean	Std deviation	F	P
3	80	275,87	183,27	1,263	0,287
3-5	13	368,14	201,67		

>5	8	349,3	201,67		
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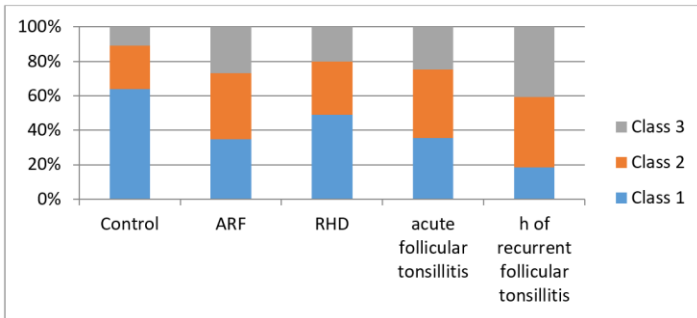


Figure 1: Class d'ASO titer in the five groups. Class 1: 200 UI/ mL, Class 2: 200-400 UI/ mL, Class 3: >400 UI/ mL.

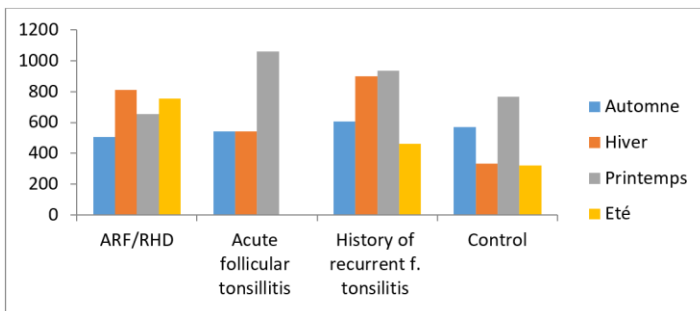


Figure 2: The upper limit of normal of ASO titer in IU/mL in different season.

The table 3 shows the variation of the ULN in each study group during 1 year of follow-up. The ASO rates are high in all groups at the time of sampling and it's still high for several months. For the RHD group, the rates of ASO still elevated until 9 months, after this period, it starts to decrease. Children with RHD adhere to monitoring, which is why the rate is well specified in this group (Table 3). The ULN is higher in boys with ARF/RHD and a history of acute recurrent follicular tonsillitis. While ASOT is higher in girls of the control group. Data of ASOT according to age class is presented in the table 4. There was a statistically significant difference of ASOT between age subgroups in all studied groups (P=0.0001) (Table 4).

The figure 2 and the table 5 present the ULN of ASO depending of the season in the studied groups. It is higher in the cold period in all groups, especially in winter and spring (p <0.001) (Figure 2) (Table 5).

There have not a statistically significant difference between patients with carditis, arthritis or chorea (p >0.05).

Within the group of children with tonsillitis, we don't find any significant correlation of ASOT with the number of attacks of tonsillitis (r=-0.13, =0.287) (Table 6).

Discussion

Group a Streptococcus infections and their sequelae are still prevalent in Morocco, hence the necessity for a precise clinical diagnosis and laboratory confirmation of GAS infections. Although there have been many studies of streptococcal antibody titers in children in different parts of the world, we are ignorant of similar data from children in our country. The present study for the first time attempts to establish the ULN values for ASO titers and their variation according to age and season for the population inhabiting Nord-EST of Morocco. In our study, the ULN values of ASO in the control group were 421, 4 IU/mL. Our results are similar of those reported in Korea, Ethiopia and Egypt (326 UI / mL, 360 UI / mL et 400 UI / mL respectively) [12]. While the ULN of ASOT in our normal healthy children was higher than that found in the study conducted in 23 different American states (240 IU/mL), in India (262 IU/mL in New Delhi, 305 IU/mL in Mumbai and 239 in Chandigarh) and in Yemen (276.2 UI/mL) (9,15–18). The majority of these values exceeded the normal level of ASO titer established by laboratories which is 200 IU/mL. Thus, for different populations in different geographic parts of the world, there may be significant differences in the ULN of ASO titer. This can be attributed to the weather or socio-economic conditions of each country. In Morocco, an ASO value greater than 400 IU / mL can be considered as a pathological value for children. In the present study, the ULN of ASOT in children with a history of repeated tonsillitis more than three times of year for the past 3 years was 756, 2 IU/ml. This was raised than that of the control group. Our results are lower than that found in the study done in Egypt by Kotby et al (1600 IU/mL) [13]. Although the levels of ASO are high, none of the children received any medical treatment because they had no complaints at the time of sampling. The re-infection can explain the elevate titers of ASO in this group of study. Some studies are mentioned that repeated exposition to different groups of streptococcus and especially GAS usually results a continuing rising of ASO levels [14]. In the tonsillitis group, the ULN of ASO titer was 561.8 IU/ml. This value is lower than found in children with a history of repeated tonsillitis. This is can be explained by the fact that just 6 out of 101 children were GAS tonsillitis which influences the level of ASO titers.

In the RHD group, the ULN above which we can diagnose streptococcal infection was 580.2 IU / mL. This value is higher than the one found in Egypt and India (200UI/mL and 262 UI/mL respectively) [15]. The increase of the ULN of ASO titer in this study group may be due to the time period between streptococcus infection and the onset of carditis that allows antibodies to reach its maximum level (3 to 6 weeks). Despite the favorable effect of penicillin on the immune response to streptococci and the

children compliance of penicillin prophylaxis for several years, the ULN of ASO in children with rheumatic heart disease was higher than that of the control group (421,4 IU / mL). This confirms that carditis has no influence on ASO titers and that prophylaxis does not necessarily lead to a normalization of these rates. Our result is confirmed by Roodpeyma and coworkers that found that carditis are not related to ASO titer. Contrarily, the study was done by Kotby and al reported an ASOT in patients with chronic RHD lower than the titer in the normal population. This is attributed to the effect of penicillin on the immune response to streptococci [16,17]. The ULN of ASOT was 829,6UI/mL in the ARF group. It is higher than the titer in the RHD children (580.2UI/mL). There is not a statistically significant difference between ASO in patients with and without carditis. So, there is no relation between carditis and the increase of ASO titers. This increase of ASO titers in this group can be explained by the effect of some concomitant disease (Cholestatic liver diseases, inflammatory rheumatism...) on the levels of ASO. In regards to the ULN of ASOT depending gender, we found that the ULN was 384.5 UI/mL in boys and 432 UI/ mL in girls. This result is higher than the one found in Yemen (252.5 UI/ mL in boys and 258.5 UI/ mL in girls) [18]. Our study shows that the high titer of ASO is related to age and there was a significant difference in ASOT according to age in all groups. The ULN is higher in children aged between 5 and 15 years with acute follicular tonsillitis (573, 25 UI/ mL), history of recurrent follicular tonsillitis (761 UI/ mL), ARF (641,95 UI/ mL) and RHD (738,6 UI/ mL). While it is lower in healthy children (419 UI/mL). This relation between age and the high levels of ASO was mentioned in several studies like in USA, India and Korea [19-26]. Our results are similar to those of Kotby and all, who found that the ULN of ASO is higher in children over 10 years of age and who did not have a recent streptococcal infection. In addition, our results are confirmed by Danchin et al, that found that the ULN of ASLO is higher in the 6-9 years and 10-14 years age group (480 IU / mL and 320 IU / mL respectively) [27]. The antibody titers vary according to season. In our study, the ASO titers are higher in the winter and spring months in all studied groups. This seasonal variation has also been demonstrated in several studies. The study done in Egypt showed that ASOT are significantly higher in autumn and winter in the control group, RHD group and the history of recurrent tonsillitis group. Another study conducted by Shet and al reported that titers of ASO was high in winter and early spring. The Indian study showed that ASO titers in children with acute rheumatic fever was higher in winter and rainy season. It is not surprising that ASO titers are higher during these seasons because streptococcal throat infections occurs more during winter and spring [28-31].

Study Limitations

More number of study children in different groups may give more significant results. The monitoring of ASO titers for one year was difficult, especially for the children of the control group and the children of history of recurrent tonsillitis groups.

Conclusion

The ULN of ASOT in normal Moroccan children is quite high, exceeding 400 IU/mL. The ULN of ASO in children with a history of recurrent tonsillitis and children with ARF / RHD is higher. Therefore, the value for positive ASOT in Moroccan children should be raised as the ULN in Moroccan children is higher than the value stated in Jones Criteria. Also the ULN is higher in boys with ARF/RHD and H of acute recurrent f. tonsillitis. While ASOT is higher in girls of the control group. Levels of ASOT increase in the age 5-15 years without affected the peak level during acute streptococcal infection. The ASO titers show the highest value in winter and spring season. Prophylaxis do not necessarily lead to a normalization of the ASO rates, because the ULN of ASOT was higher in patients with carditis.

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Conflicts of interest statement

The authors declare that they have no conflicts of interest

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